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13. ABSTRACT (Maximum 200 Words)

The current project proposes to develop a novel ultrasound contrast imaging technique (called EEI) for better visualization of the microvessels, which are characteristic of the neovasculature associated with prostate cancer.

The new zero-thickness interface model was used to simulate EEI. While results at an imaging frequency of 7.5 MHz were in reasonable agreement with measurements (3-4 dB enhancement), there was no agreement for 3 MHz imaging (10 dB measured but no enhancement simulated). Further work is ongoing to improve upon the model. The pulse-echo system was used to perform in vitro EEI measurements at 7.5 MHz. Around 6 dB of enhancement was measured with Optison irrespective of temperature, whereas Sonazoid produced 6 dB of enhancement at 22° C and only 3 dB at 37° C. Finally, a Logiq 9 scanner with a 3.5C curved linear array and an AN2300 digital ultrasound engine with a P4-2 phased array transducer were modified to perform EEI on a vector-by-vector basis in fundamental and pulse inversion harmonic grayscale modes. A flow phantom with contrast was imaged in vitro. While video intensities of scattered signals from the surrounding tissue were unchanged, video intensities of echoes from contrast bubbles within the vessel were markedly enhanced. The maximum enhancement achieved was 10.4 dB in harmonic mode.

14. SUBJECT TERMS
Prostate Cancer, Ultrasound Imaging, Ultrasound Contrast Agent

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4. INTRODUCTION

The diagnosis of prostate cancer is currently based on an elevated prostate-specific antigen (PSA) level or abnormal digital rectal examination findings confirmed by needle biopsy of the prostate. It is estimated that the number of men subjected to biopsy of the prostate in the U.S. in 2001 exceeded 600,000 [1]. Unfortunately, the frequency of positive biopsy findings, for most screening populations, was as low as one in three to one in four. Therefore, an accurate, noninvasive diagnostic imaging examination of the prostate is needed to reduce the number of biopsies or even to replace biopsy.

A sextant biopsy of the prostate, consisting of the acquisition of six biopsy cores, can miss clinically detectable prostate caner in up to 34% of men. Among patients with an elevated PSA level and a negative initial sextant biopsy finding, repeat biopsy demonstrates the presence of malignancy in approximately 20%-30% [2]. However, each additional biopsy is associated with a small incremental risk of hemorrhage and infection. Thus, an accurate, noninvasive imaging technique is useful for targeted biopsy guidance in order to reduce the number of biopsies in each prostate.

The sensitivity and specificity of ultrasound imaging can be improved by intravenous injection of vascular contrast agents consisting of encapsulated gas microbubbles [3]. Due to encapsulation, these agents are stable enough to pass through the pulmonary circulation and flow in intravascular space for at least several minutes. However, encapsulation imposes severe restrictions on the oscillations of contrast bubbles. Based on de Jong's numerical model [4], our calculations indicate that the incident acoustic pressure amplitude for an albumin-encapsulated Abunex® bubble is 18 times greater than that for a free bubble if the two bubbles oscillate with the same relative amplitude at their resonant frequency of 2 MHz. Hence, scattering can be greatly enhanced if encapsulated bubbles become free bubbles. According to the Rayleigh's approximation, the fundamental scattering cross-section of an air bubble is more than 200 times (47 dB) greater than that for an Abunex bubble of the same size. Furthermore, the enhancement in second or sub- harmonic scattering cross-section must be even much greater because encapsulation dampens nonlinear oscillations to a much greater degree.

Unlike current ultrasound imaging modalities employing only an imaging field, the proposed technique utilizes two acoustic fields: the activation field for intermittently activating contrast bubbles and the imaging field, applied shortly afterwards, for acquiring harmonic or subharmonic images with significantly enhanced scattering signals from activated contrast bubbles. This new imaging mode is referred to as Excitation Enhanced Imaging (EEI) [5]. Based on previous work on ultrasound-induced contrast scattering enhancement and contrast-assisted ultrasonographic detection of human and canine prostate cancer, the hypotheses of this study are: (1) contrast microbubbles can be ultrasonically activated to achieve marked backscattering enhancement, and (2) the detection of prostate cancer can be improved using the proposed ultrasonographic technique. It is well known that a free bubble resonating at the insonation frequency is the optimal acoustic scatterer. Hence, the activation field will consist of a release pulse for effectively releasing free bubbles from encapsulated contrast bubbles and an excitation pulse for shifting the free bubbles to the resonance size corresponding to a pre-selected imaging frequency.

5. BODY

It is the central hypothesis of this study that contrast microbubbles can be activated and backscattering enhanced markedly. The activation field will produce the optimal acoustic scatterers, i.e., free bubbles resonant around a pre-selected imaging frequency. In the proposed study, both the modeling and measurement will help us to determine optimal contrast agents and develop optimal activation pulse sequences enhancing backscattered second and sub-harmonic signals by a factor of 2 to 16, i.e., by 6 to 24 dB. The other hypothesis of this study is that prostate cancer can be detected using the proposed ultrasonographic technique. This hypothesis will be tested using an established canine prostate tumor model. The specific tasks of the project (as presented in the original Statement of Work) can be found in Appendix I.

First an outline of the methods applied will be given followed by a presentation of the results to date. Finally, the conclusions and future directions of the research will be discussed.

5.1 Methods

Contrast microbubble modeling
We have adopted a Newtonian rheology, i.e., only viscous interfacial stresses are considered (see [6] for details) to develop a new simulation model of contrast bubbles based on a modified Rayleigh-Plesset type equation (as detailed in the previous report and in [7]):

$$\rho(R\ddot{R}+\frac{3}{2}\dot{R}^2) = P_{G0}\left(\frac{R_0}{R}\right)^{3k} - 4\mu\frac{\dot{R}}{R} - \frac{2\gamma}{R} - \frac{4\kappa^s\dot{R}}{R^2} - P_0 + P_A\sin\omega t \qquad (1)$$

The bubble radius is given by ($r = R$), while $p(r=R,t)$ is the pressure in the liquid immediately outside of the bubble, P_G is the uniform gas pressure inside the bubble, μ the liquid viscosity, κ^s and γ are interfacial dilatational viscosity and surface tension respectively. $\dot{R}$ is the bubble wall velocity while R is the instantaneous bubble radius.

Dual pulse EEI with Sonazoid (using size distribution and attenuation data from [8, 9]) was simulated. The PRF used was 2 Hz while 36 and 16 cycles were used for imaging and excitation signals, respectively, with acoustic pressures of 0.1 and 1.2 MPa (i.e., similar to the parameters used for the *in vitro* measurements). The excitation pulse frequency employed was 1.1 MHz and the imaging frequency was either 3.0 MHz or 7.5 MHz. This represents the continuation of task 1c (see original SOW in the Appendix).

In Vitro experiments
A system was built to perform EEI and measure the enhancement of scattered signals from contrast microbubbles in a water bath with three single-element spherically-focused transducers (Staveley, East Hartford, CT, USA), as shown in Figure 1. An excitation transducer (1.1 or 2.1 MHz) with a diameter of 2.5 cm and a focal length of 5.0 cm was used for conditioning microbubbles and a pair of small broadband imaging transducers (with center frequencies of 3

and 7.5 MHz), for detecting these microbubbles before and after conditioning. The excitation transducer was driven by a programmable arbitrary function generator (LW420; LeCroy, Chestnut Ridge, NY, USA) through a 500 W power amplifier (A-500; ENI, Rochester, NY, USA). One imaging transducer was used to transmit imaging pulses produced by a programmable function generator (8116A; Hewlett Packard, Santa Clara, CA, USA) and a broadband power amplifier (325LA; ENI) and another was employed to receive signals scattered from the contrast microbubbles. The scattered signals were amplified and then acquired using a digital oscilloscope (9350AM; LeCroy, Chestnut Ridge, NY, USA). A time modulus (AV-1023-C; Avtech Electosystems, Ogdensberg, NY, USA) was used to synchronize the delay between the conditioning and imaging pulses. The command delivery to the function generators and the data transfer from the digital oscilloscope were controlled by LabView® (National Instruments, Austin, TX, USA).

Four different types of contrast agents were tested: a) Sonazoid® (GE Healthcare, Oslo, Norway), a lipid-coated contrast agent containing a PFC gas; b) Optison® (GE Healthcare, Princeton, NJ), an albumin-encapsulated agent filled with perfluorobutane; c) BG1135 (Bracco Research SA, Plan-les-Ouates, Switzerland), an experimental, polymeric shell agent consisting of air-filled microbubbles; and d) QFX (Nanfang Hospital, Guangzhou, China), made up of PFC microbubbles stabilized with albumin coating. During the experiments, contrast agents were diluted in water (e.g., around 20 µl of reconstituted Sonazoid per liter of water) and a magnetic stirrer was used to maintain mixture. Excitation amplitudes of 0.4, 0.8, 1.2 and 1.6 MPa were investigated along with excitation pulse lengths of 2 to 35 cycles operating at pulse repetition frequencies (PRFs) of 2, 10 and 20 Hz. Inter-pulse delays from 10 to 750 µs were studied.

In vitro experiments were conducted at ambient temperature (22° C) and physiological temperature (37° C). For each enhancement measurement, spectra of scattered imaging signals from unconditioned and conditioned contrast microbubbles were acquired. The average spectrum for regular contrast microbubbles (before conditioning) was obtained at a given PRF based on a sequence of 64 scattered signals from transmit imaging pulses. The same imaging pulse was transmitted with a given delay after each condition pulse for 64 times (at the same PRF), so that the averaged spectrum for the conditioned microbubbles was obtained. This represents the continuation of task 1d and the commencement of task 1e (see original SOW in the Appendix).

Two different scanning platforms were selected for initial implementation of real-time EEI. One platform was the state-of-the-art, all digital, flagship ultrasound scanner from GE: the Logiq 9 (GE Healthcare, Milwaukee, WI). The second platform selected was an AN2300 digital ultrasound engine (Analogic Corporation, Peabody, MA). This is a development platform featuring a 64 channel digital beamformer front end with continuous dynamic receive focus and apodization, a 40 MHz vector processor, scan converter, real-time controller, and a PC system host with a Windows NT operating system. The AN2300 comes with high level software toolboxes allowing acquisition, analysis and display to be implemented and controlled through ANSI C and C++ routines.
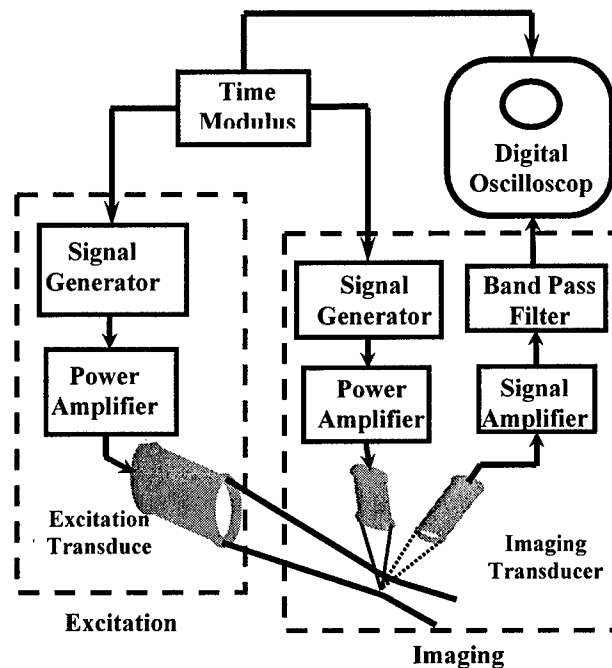
Figure 1. *Experimental set-up for EEI enhancement measurement. An excitation transducer was used to condition contrast microbubbles and a pair of broadband transducers (one for transmission and another for reception, perpendicular to each other and to the excitation transducer) were employed to detect the conditioned microbubbles.*

In both scanners the EEI software modifications supported real time and intermittent frame rates operating in fundamental as well as pulse inversion HI grayscale EEI modes. Moreover, EEI was implemented to enable the transmission of an excitation pulse followed by an imaging pulse (or 2 imaging pulses in the case of pulse inversion HI) on a vector-by-vector basis. This transmission mode is similar to standard color Doppler firing modes (albeit with only 2-3 firings along each scan line), which made implementation easier. The standard signal processing chain was then used to produce real time EEI.

Both the Logiq 9 and the AN2300 scanners were successfully modified to incorporate the necessary software changes enabling the transmission of an excitation pulse followed by an imaging pulse (or two for pulse inversion HI) on a vector-by-vector basis (this work was supported in parts by NIH HL62830). Following the modifications, EEI could be performed in fundamental as well as pulse inversion HI grayscale modes at frame rates of either 1 or 30 Hz (i.e., intermittent or real time EEI). As of today we have not identified an endocavitary probe with sufficiently broad bandwidth to accommodate both low frequency excitation pulses and high frequency (> 7 MHz) imaging pulses. Hence, two broad bandwidth transducers, the 3.5C and the P4-2 (Philips Medical Systems, Bothell, WA) were selected for the Logiq 9 and the AN2300, respectively, because of their ability to accommodate the requirements for low frequency excitation pulses (< 3 MHz) and comparable or higher frequency imaging pulses [5]. The excitation pulse employed on the Logiq 9 was a 1.6 MPa, 4 cycle, 2.5 MHz pulse. The AN2300 utilized a longer, lower frequency excitation pulse (12 cycles at 2.0 MHz) to

compensate for the lower pulse amplitude of 1.1 MPa. Both scanners were configured to transmit and receive at 2.2 and 4.4 MHz, respectively, in pulse inversion HI mode, while the Logiq 9 operated at 3.6 MHz and the AN2300 at 3.2 MHz in fundamental EEI mode. The delay between the excitation and the imaging pulse was previously found to have limited effect on the enhancement produce in EEI mode [5] and was selected to be 375 μs and 100 μs on the Logiq 9 and the AN2300, respectively. This represents the beginning of task 2c as outlined in the original SOW (see the Appendix).

To demonstrate the contrast imaging enhancement achievable with EEI *in vitro*, a tissue-mimicking flow phantom with an 8 mm vessel embedded (Model 524; ATS laboratories, Bridgeport, CT) was imaged. During the experiments, contrast agents were diluted in water (e.g., around 20 μl of reconstituted Sonazoid per liter of water) and a magnetic stirrer was used to maintain mixture while the diluted microbubble suspension was pumped through the flow system by a continuous flow roller pump (S10K II; Sarns Inc., Ann Arbor, MI). The amplitude of the excitation pulses was varied from maximum (0 dB) to minimum (-20 dB i.e., no excitation pulse) and EEI was performed in fundamental as well as harmonic mode. Digital cine-clips and images were recorded before and after transmission of the excitation pulses and transferred to a PC for off-line analysis. The mean video intensity in a region of interest (ROI) corresponding to the vessel was determined using Image-Pro Plus software (Media Cybernetics, Silver Spring, MD) and the signal-to-noise-ratio (SNR) relative to baseline (i.e., no excitation pulse) was calculated in dB. The effect of excitation pulse amplitude on the SNR and, thus, on the EEI enhancement, was analyzed statistically and compared using a one-way analysis of variance (ANOVA; Matlab, The Mathworks Inc, Natick, MA) with p-values less than 0.05 considered significant. The SNR was the dependent variable, while amplitude was considered the independent variable. This represents the initiation of tasks 2c and 2d as outlined in the original SOW (see the Appendix) and has been submitted as a paper to a peer-reviewed journal [10].

5.2 Results and Discussion

The ability of our novel simulation model [7] to simulate the dynamic behavior of microbubbles during EEI was examined (using parameters $\kappa^s = 0.01$ msP and $\gamma = 0.6$ N/m). The excitation and imaging pulses used are depicted in Figure 2 (for two different inter-pulse delays). At an imaging frequency of 3 MHz the model was not able to reproduce the experimental results and did not show an effect due to the excitation field. Moreover, for the short inter-pulse delay of 10 μs the model simply adds the response of the excitation and the imaging pulses, which does not correspond to the experimental results obtained *in vitro* [5, 10]. However, there was a small enhancement of 4 dB when the imaging frequency was increased to 7.5 MHz (for a delay of 100 μs), which appear to be caused by ringing effects produced by the excitation pulse. Currently, efforts are ongoing to produce a more realistically model incorporating bubble growth via rectified diffusion.

The change in scattered signal strength before and after the excitation pulse (i.e., the enhancement obtained with EEI relative to standard contrast imaging) was measured under different acoustic conditions using the pulse-echo system outlined in Figure 1. In Figure 3a, an example based on measurements obtained at 7.5 MHz (i.e. a realistic imaging frequency for the

(a)   (b)

8
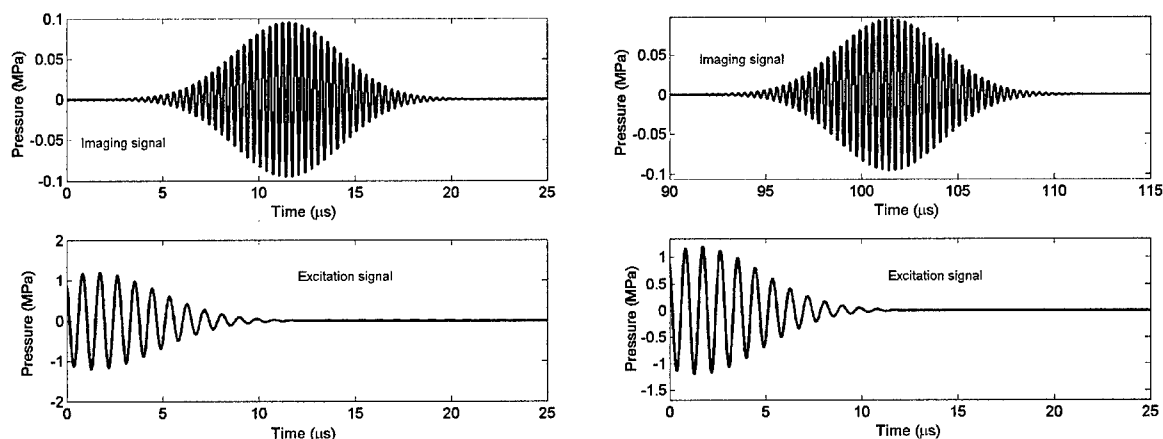
Figure 2. *Simulated excitation and imaging pulses for EEI with Sonazoid. The inter-pulse delays were (a) 10 μs and (b) 100 μs.*

prostate) with Sonazoid at 22° C is shown. EEI induces scattering enhancement with a maximum of approximately 6 dB around 7.5 and 15 MHz (the fundamental and second harmonic frequencies) for Sonazoid microbubbles conditioned with 1.2 MPa excitation pulses (1.1 MHz and 16 cycles) at 2 Hz PRF. Imaging pulses (36 cycles, 7.5 MHz and 0.1 MPa) were transmitted, with a delay of 100 μs between the conditioning pulse and the detection pulse. However, when the temperature was elevated to the physiological level (i.e., 37° C; Fig. 3b) enhancement reduced to around 3 dB. EEI with Optison (employing the same conditioning and imaging conditions) produced very different results. Figure 4 demonstrates almost the same enhancement at 22° C as at 37° C (approximately 7 and 3 dB at the fundamental and second harmonic frequencies, respectively). These results are very different from the ones reported in the last report with an imaging frequency of 3 MHz, where Optison produced almost no enhancement at room temperature but almost 10 dB at 37° C. Clearly both temperature and imaging frequency are important parameters for EEI, but the impact varies with contrast agent.

Examples of images recorded in the cine loop of the Logiq 9 scanner before and after transmission of the excitation pulse at a 1 Hz frame rate (i.e., intermittent EEI) are given in Figure 5. Conventional pulse inversion HI of BG1135 in Figure 5a shows the vessel poorly, because the contrast echoes are *en par* with the tissue echoes. Following application of the excitation pulse, a marked enhancement in contrast signals within the vessel, but a minimal enhancement in tissue echoes, is observed (Figure 5b). The change in SNR obtained with EEI relative to standard contrast imaging was measured as a function of excitation pulse amplitude. In Figure 6, an example based on measurements with BG1135 imaged in HI mode is shown. The maximum improvement in SNR was 10.4 dB. On average the improvement was 6.3 dB ± 1.29 dB, which was measured for a -4 dB excitation pulse amplitude (cf., Figure 6). The ANOVA showed the effect of EEI was significant ($p = 0.0007$). In fundamental mode the best SNR measured with QFX was 7.8 dB, while the mean improvement in SNR was found to be 4.1 dB ± 0.13 dB for a -6 dB excitation amplitude ($p < 0.0001$). These SNR improvements are somewhat
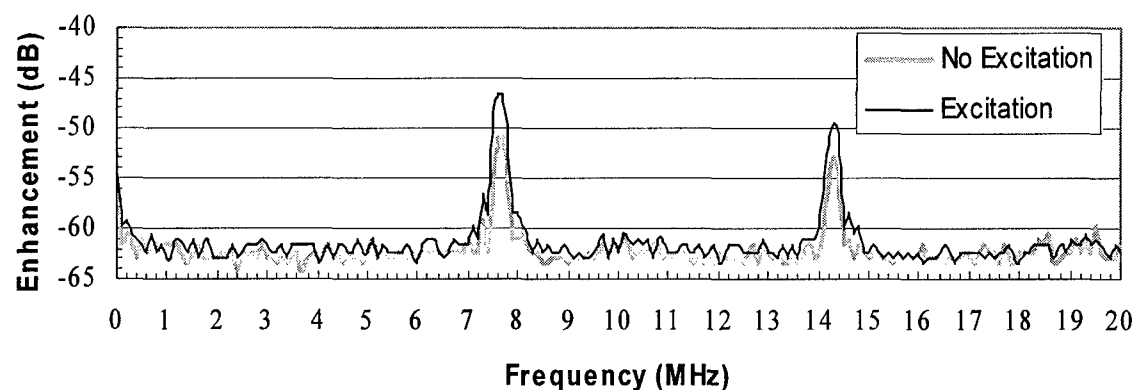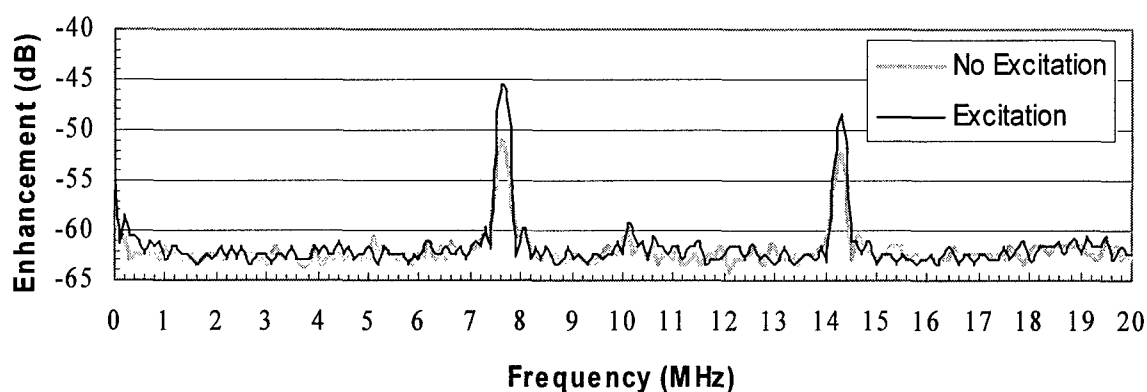
(a)

9
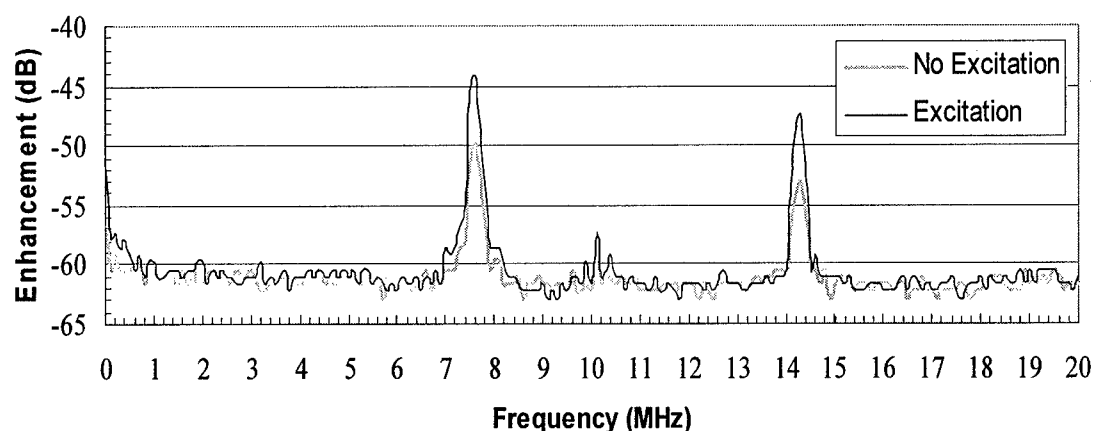
(b)
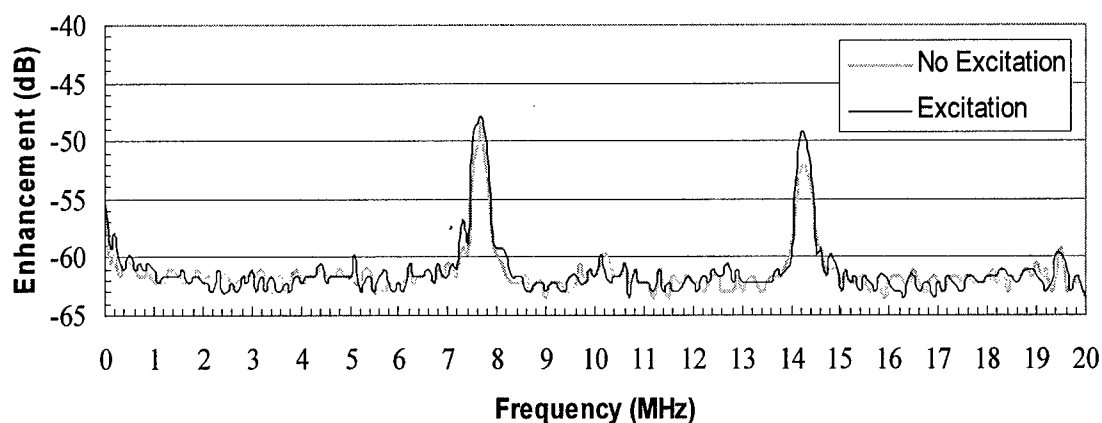
Figure 3.   *Measurement of enhancement from Sonazoid in EEI mode using 1.1 MHz excitation at 1.2 MPa, imaging at 7.5 MHz, 2 Hz PRF obtained at (a) 22° C and (b) at 37° C. Notice, that no enhancement is seen at the physiological temperature (whereas marked enhancement ~6 dB occurs at room temperature).*

lower (by 4 – 6 dB) than those measured with the EEI pulse-echo set up [5]. Most likely this is caused by the flow of conditioned microbubbles out of the imaging plane and the differences in transducer sensitivity that occur when single element transducers are replaced by broad bandwidth arrays.   Moreover, the slightly reduced enhancement observed at the highest excitation amplitudes in Figure 6 is probably due to bubble destruction i.e., destroyed microbubbles were not completely replenished between excitation pulses. Thus tasks 2c and 2d as outlined in the original SOW (see the Appendix) have been initiated. Since these experiments did not involve the final choice of transducers it was decided to temporarily bypass tasks 2a and 2b.

(a)

10

Figure 4. *Measurement of enhancement from Optison in EEI mode using 1.1 MHz excitation at 1.2 MPa, imaging at 7.5 MHz, 2 Hz PRF obtained at (a) 22° C and (b) at 37° C. Notice, that almost the same enhancement is seen at room temperature as at the physiological temperature.*

6.   KEY RESEARCH ACCOMPLISHMENTS

- The new zero-thickness interface model was expanded to describe the dual pulse mode of EEI, but further work is necessary.
- A dual-transducer pulse-echo system was used to perform EEI.
- Initial experiments were conducted at 3.0 and 7.5 MHz with 2 contrast agents.
- Temperature dependencies were studied.
- Up to 6.9 dB of enhancement was measured at 22° C for Optison when imaging at 7.5 MHz.
- An initial version of EEI software was implemented on two ultrasound scanners.
- *In vitro* flow phantom measurements with 3 contrast agents demonstrated maximum enhancement of 10.4 dB in harmonic mode (mean enhancement: 6.3 dB; p = 0.0007)

(a)   (b)

11
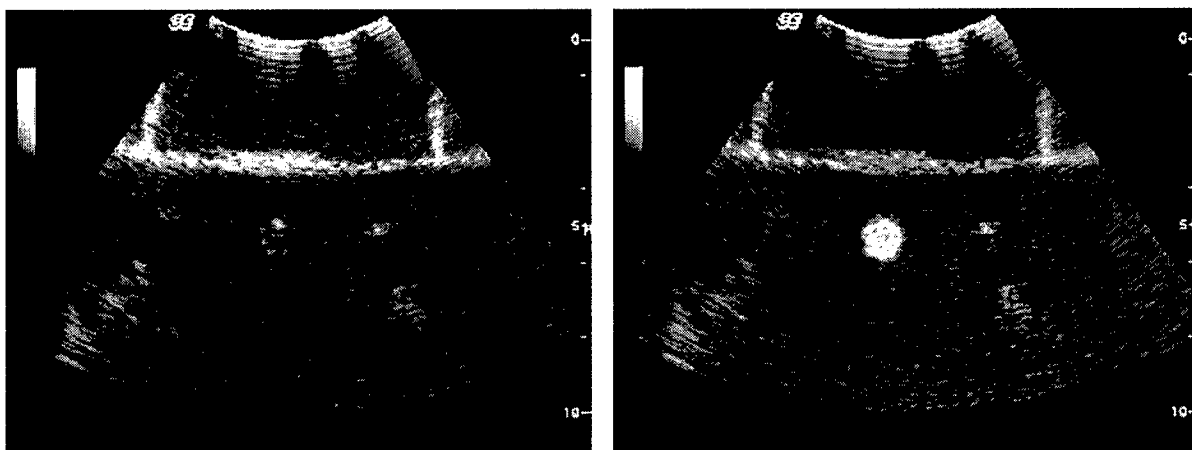
Figure 5. *Intermittent, pulse inversion HI of BG1135 obtained with the Logiq 9 scanner (a) without EEI and (b) with EEI. Notice the marked improvement in SNR within the vessel following activation of EEI.*
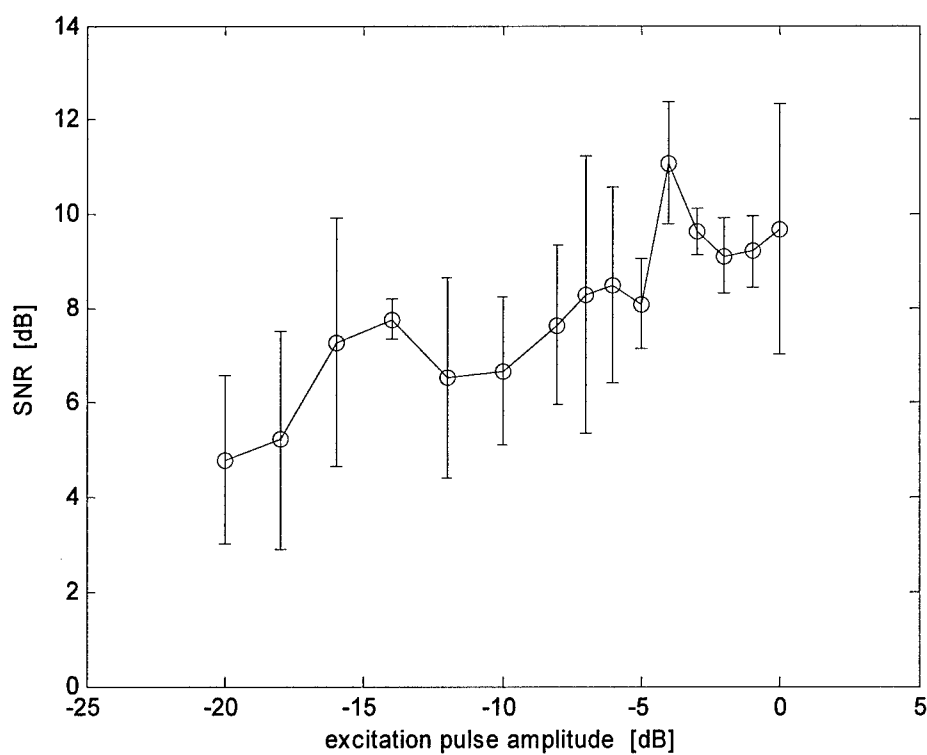
Figure 6. *Changes in SNR as a function of excitation amplitude for BG1135 imaged in HI mode. The -20 dB amplitude corresponds to conventional HI (i.e., baseline without EEI).*

7. REPORTABLE OUTCOMES

R. J. Ro, F. Forsberg, M. Knauer, W. T. Shi, P. A. Lewin, R. Bernardi. On the temperature and concentration dependency of excitation-enhanced imaging. *Proc Biomed Eng Soc Ann Fall Meetg*, abstract no. 1059, 2004.

K. Sarkar, W. T. Shi, D. Chatterjee, F. Forsberg. Characterization of ultrasound contrast microbubbles using in vitro experiments and viscous and viscoelastic interface models for encapsulation. *J Acoust Soc Am,* vol. 118, 2005. In press.

F. Forsberg, W. T. Shi, M. M. Knauer, A. L. Hall, C. Vecchio, R. Bernardi. Real time excitation enhanced ultrasound contrast imaging. Submitted to *Ultrasonic Imaging*, February, 2005.

October 13-16, 2004   Biomedical Engineering Society Annual Fall Meeting, Philadelphia, PA, USA.
- On the temperature and concentration dependency of Excitation-Enhanced Imaging.

8. CONCLUSIONS

The new zero-thickness interface model was used to simulate the dual pulse imaging mode associated with EEI. While results at an imaging frequency of 7.5 MHz were in reasonable agreement with measurements (3-4 dB enhancement), there was no agreement for 3 MHz imaging (10 dB measured but no enhancement simulated). Further work is ongoing to improve upon the model.

The pulse-echo system was used to perform *in vitro* EEI measurements at 7.5 MHz and initial experiments were conducted with 2 contrast agents. Around 6 dB of enhancement was measured with Optison irrespective of temperature, whereas Sonazoid produced 6 dB of enhancement at 22° C and only 3 dB at 37° C.

Finally, a Logiq 9 scanner with a 3.5C curved linear array and an AN2300 digital ultrasound engine with a P4-2 phased array transducer were modified to perform EEI on a vector-by-vector basis in fundamental and pulse inversion harmonic grayscale modes. Ultrasound contrast microbubbles within an 8 mm vessel embedded in a tissue-mimicking flow phantom were imaged *in vitro*. While video intensities of scattered signals from the surrounding tissue were unchanged, video intensities of echoes from contrast bubbles within the vessel were markedly enhanced. The maximum enhancement achieved was 10.4 dB in harmonic mode (mean enhancement: 6.3 dB; $p = 0.0007$).

In summary, task 1 has been almost completed while task 2 is ongoing, but due to the delay caused by the issues with the simulation model as well as the variabilty of EEI with temperature, imaging frequency and contrast agent the project is approximately 6 months behind schedule.

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Appendix I

The Statement of Work from the original proposal:

Task 1: To investigate activation-induced scattering enhancement at different center frequencies, amplitudes, and shapes (or lengths) of activation pulse sequences (Months 1-18):

    a. Construct an *in vitro* experimental system for ultrasonically activating contrast microbubbles and measuring the resulting changes in backscattering (Months 1-2).

    b. Design and develop numerical codes for a theoretical model describing the dynamics and instability of ultrasonically activated contrast microbubbles (Months 1-6).

    c. Calculate the behavior of individual contrast microbubble and the collective behavior of contrast microbubble populations (Months 6-18).

    d. Measure changes in backscattered fundamental, second and sub-harmonic signals before and after activation (Months 3-18).

    e. Predict optimal contrast agents for ultrasound-activated contrast imaging according to the numerical simulations (Months 12-18)

    f. Select optimal contrast agents for ultrasound-activated contrast imaging. The selection is mainly based on experimental measurements (Months 12-18).

    g. Develop activation and imaging strategies, based on both numerical simulations and experimental measurements for the scattering enhancement (Months 12-18).

Task 2: To implement ultrasound-activated contrast imaging (Months 18-24):

    a. Produce and evaluate activation-enhanced A-lines in an *in vitro* perfusion phantom using the simple pulse-echo system (Months 18-20).

    b. Optimize activation and imaging strategies, based on *in vitro* phantom measurements and simulations with actual parameters of designated transducers (Months 18-24).

    c. Modify a state-of-the-art ultrasound imaging system to incorporate the ultrasound-activated contrast imaging modality (Months 21-24).

    d. Evaluate the new imaging modality in an *in vitro* perfusion phantom using the modified ultrasound scanner (Months 21-24).

Task 3: To validate the clinical potential of ultrasound-activated contrast imaging using an established canine prostate cancer model (Months 25-32):

    a. Create and grow prostate tumors by implanting a Canine Transmissible Venereal Sarcoma (CTVS) cell line into the prostate (Months 25-29).

    b. Produce and evaluate activation-enhanced contrast images of canine prostates with CTVS tumors (Months 26-30).

    c. Perform pathological evaluations of prostate specimens and quantify the microvessel density with immunohistochemical staining (Months 28-31).

    d. Process data and images and write final report (Months 31-32).